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Hereditary Molecular Diseases (Inborn Errors)

W. EUGENE KNOX*

The most striking property of hereditary molecular diseases is that they reveal to us with great nicety one part of the machinery of life, often one we did not know existed while it smoothly performed its function. These are molecular ablations which have a precision far greater than the relatively crude removal of a whole organ such as an endocrine gland, and even greater than metabolic blocks caused by vitamin deficiencies or antimetabolites, which may affect a whole series of related reactions. Over the years these diverse, bizarre, and often rare "experiments of nature" have been observed and recorded. Knowledge about them slowly cumulated, often garnered by people with the devotion of the true amateur. Such a man was Sir Archibald E. Garrod. He was the first person to grasp the idea of an hereditary control of the individual steps in metabolism which resulted in what he called the inborn errors of metabolism. More recently the fusion of the natural sciences, especially biochemistry and genetics, with molecular chemistry and physics has made possible an even grander conceptualization of this entire area, from the genetic information to its expression in an individual as the clinical signs and symptoms of disease.

One consequence of this advance has been the successful introduction of the preferable term for these conditions: the hereditary molecular diseases. At present the guiding intention is to couch all aspects of the disease, both its transmission and its manifestation, in molecular terms, i.e., as properties of the structure and function of chemical molecules. The historical development of this concept best illustrates the extraordinary accomplishments which are now being realized. This can be traced through the particularly illuminating studies of only a few diseases: alkaptonuria, sickle cell anemia, and phenylketonuria.

"The Inborn Errors of Metabolism"

It is now just fifty years since the first appearance of Garrod's slim volume, "The Inborn Errors of Metabolism."[4a] But the genesis of the idea expounded in this classical discussion of four diseases (alkaptonuria, albinism, cystinuria and pentosuria) took place a few years earlier during the study of one of these, alkaptonuria, the prototype of the inborn errors of metabolism.

Alkaptonuria

The modern history and the name of this disease began in 1859 when Bödecker was sent for examination a urine sample from a 44 year old man suffering from incapacitating arthritic back pain. At first it was thought he had diabetes, because his urine reduced Fehling's solution in the standard test for

*Cancer Research Institute, New England Deaconess Hospital; Assistant Professor of Biological Chemistry, Harvard University School of Medicine, Boston, Massachusetts.

sugar. But as Bödecker alkalinized the urine preparatory to the test, unlike a diabetic urine, it turned dark from the surface down, and took up more than its own volume of oxygen gas. The substance in the urine responsible for this curious behavior he named "alkapton" from the combination of the Arabic word for alkali and the Greek word meaning "to suck up (oxygen) greedily."

Later the substance was identified as homogentisic acid*, a compound easily oxidized by air to a black polymer and chemically related to the hydroquinone used in developing photographic plates. Exposed photographic film is indeed blackened immediately by a drop of urine from an alkaptonuric patient. This is one of the simplest tests for this acid. Pieter (1925) jestingly advised that his patient with this (supposedly) harmless affliction take up photography, since he would have no need for commercial developers.[14]

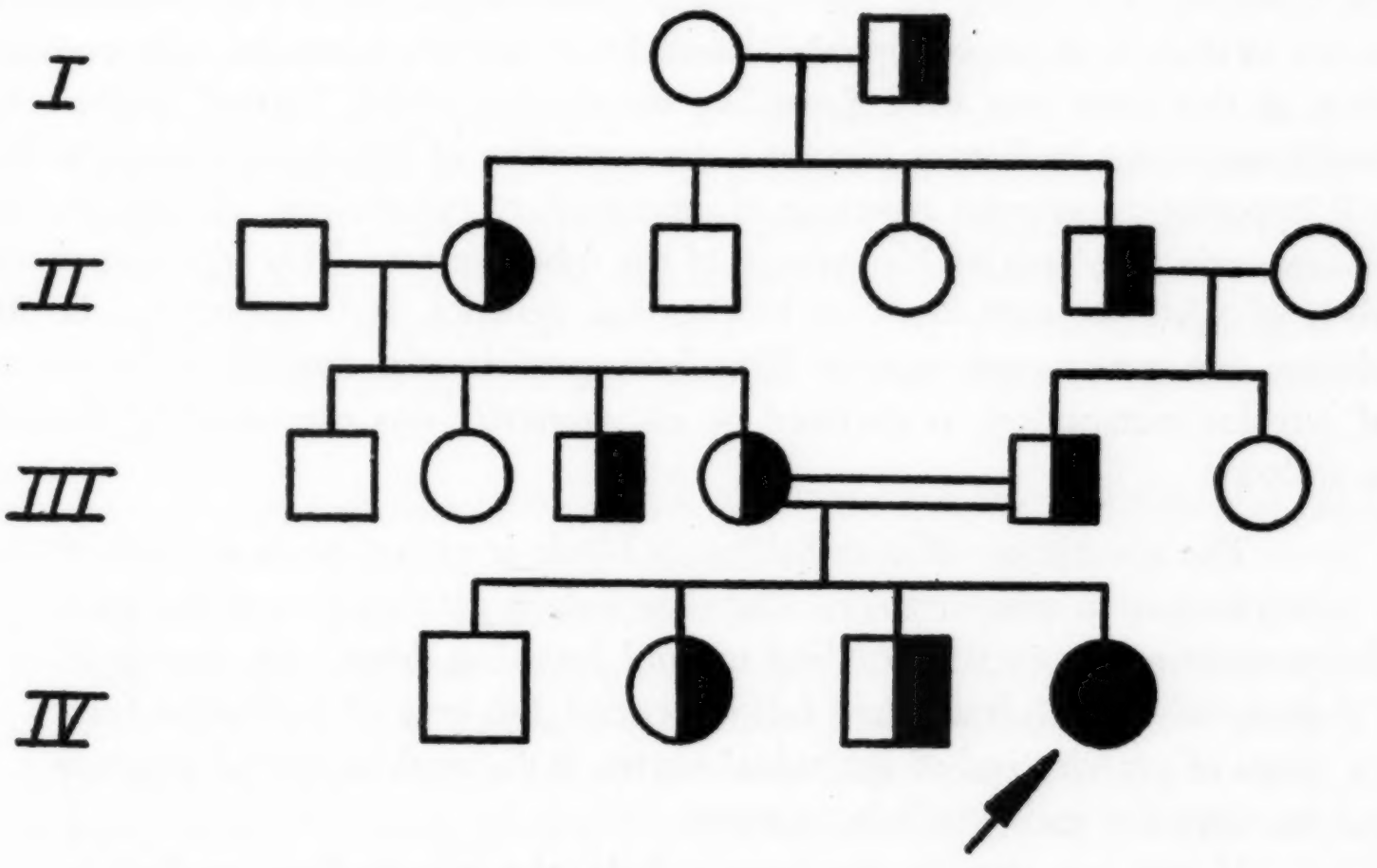
The blackening of urine upon standing and the staining of wetted clothes is a typical bizarre manifestation of this inborn error of metabolism. According to Garrod, these diseases "advertise their presence in some conspicuous way, either by some strikingly unusual appearance of surface tissues or of excreta, by the excretion of some substance which responds to a test habitually applied in the routine of clinical work, or by giving rise to obvious morbid symptoms."[4a] The reliability of such a sign is implicit in the retrospective diagnoses of this condition which Garrod made from early medical writings, and which is quoted as an example of his distinctive style:

> Until the early years of the nineteenth century, no distinction was drawn in medical writings between urines which were black when passed and such as darkened on exposure to air, but it is difficult to suggest any other diagnosis than that of alkaptonuria for some cases referred to in works of the sixteenth and seventeenth centuries, such as that mentioned by G. A. Scribonius (in 1584) of a schoolboy who, although he enjoyed good health, continuously excreted black urine, and that cited by Schenck (in 1609) of a monk who exhibited a similar peculiarity and stated that he had done so all his life. The most interesting record of this kind is to be found in the work of Zacutus Lusitanus, published in 1649. The patient was a boy who passed black urine and who, at the age of fourteen years, was submitted to a drastic course of treatment which had for its aim the subduing of the fiery heat of his viscera, which was supposed to bring about the condition in question by charring and blackening his bile. Among the measures prescribed were bleedings, purgation, baths, a cold and watery diet, and drugs galore. None of these had any obvious effect, and evenually the patient, who tired of the futile and superfluous therapy, resolved to let things take their natural course. None of the predicted evils ensued, he married, begat a large family, and lived a long and healthy life, always passing urine black as ink.[4a]

*Formerly called glycosuric acid, homogentisic acid is an intermediate in the oxidation of tyrosine and phenylalanine.

Garrod, though later the successor to Sir William Osler as the Regius Professor of Medicine at Oxford, was a pediatrician at the time he published his first paper on alkaptonuria.[2] As a pediatrician, he saw the entire family unit of his patients more clearly than physicians in other specialities are apt to do. His first paper added five cases to the 23 then known examples of alkaptonuria, and confirmed the fact that this very rare disease might be met in several members of one family. Specifically it occurred among siblings, but not in their parents or their offspring. There was "a very special liability of alkaptonuria to occur in the children of first cousins." The import of these facts was not immediately clear for Mendel's laws were not rediscovered until the following year. Garrod continued, however, to collect particular bits of information about this disease which were oddly off the main paths of current research. He chronicled, diaper by diaper, the appearance of urinary staining in a new-born child, and so established the abnormality to be of life-long duration, i.e., congenital. Feeding experiments convinced him that the amino acid, tyrosine, known to be the source of homogentisic acid, must be absorbed and converted to this product by the body's metabolism because of the time lag before the excretion began, and not as then thought, by fermentation in the gut.

FIGURE 1



MENDELIAN LAW OF HEREDITY

Typical pedigree showing the transmission of a single abnormal gene to about half the members of generations II and III, and a first cousin marriage in generation III between two carriers. The disease (arrow) may then appear in generation IV, on the average in one-fourth of the siblings and usually only in the siblings of that generation. Alternatively, the same diagram can represent the normal and abnormal complements of a specific protein in the individuals.

When Bateson, in 1902, published *Mendel's Principles of Heredity,* Garrod had gathered his already mentioned facts about alkaptonuria. It immediately became clear that a rare recessive hereditary factor of the sort postulated by Mendel could explain this congenital disturbance of metabolism: "What may be described as a 'freak' of metabolism, a chemical abnormality more or less analogous to structural malformations." This kind of heredity could explain the observed familial occurrence.

> There is no reason to suppose that mere consanguinity of parents can originate such a condition as alkaptonuria in their offspring, and we must rather seek an explanation in some peculiarity of the parents, which may remain latent for generations, but which has the best chance of asserting itself in the offspring of the union of two members of a family in which it is transmitted.[3]

In such a family, illustrated by the pedigree of Figure 1, the effects of a single "recessive" gene are not manifest. Since it is a rare gene, both parents are more likely to have it if the marriage was within the family. When this occurs, the disease appears on the average in one out of four children. It may possibly occur in several children, and only rarely would there be a family history of the same disorder in other generations. Alkaptonuria was the first human or animal condition recognized to follow the Mendelian law.

The theory of the inborn errors involved a second assumption in addition to the recessive inheritance of the chemical abnormality. This was: Metabolism occurs in discrete steps, susceptible to individual hereditary control. The popular view at that time was of a flame-like metabolism which burned brighter in health and lower in disease. Garrod's clear coupling of hereditary control to the still hypothetical enzyme reactions of intermediary metabolism was the key to alkaptonuria, the basis of his concept of the inborn errors. This represented the birth of what we now know as biochemical genetics. It is epitomized in the phrase: one gene — one enzyme. Why homogentisic acid, formed in the course of tyrosine metabolism, is excreted by alkaptonurics was explained by Garrod as follows:

> The conception of metabolism in block is giving place to that of metabolism in compartments. The view is daily gaining ground that each successive step in the building up and breaking down, not merely of proteins, carbohydrates, and fats in general, but even of individual fractions of proteins and of individual sugars, is the work of special enzymes set apart for each particular purpose.
>
> If any one step in the process fails, the intermediate product in being at the point of arrest will escape further change, just as when the film of a biograph is brought to a standstill the moving figures are left foot in air. All that is known of the course of catabolism tends to show that in such circumstances, the intermediate product in being is wont to be excreted as such.

Subsequent work has fully borne out Garrod's general ideas of heredity and metabolism and fully confirmed his particular views of alkaptonuria.[10] It is a rare disease. Estimates of incidence range from one in 250,000 to one in 10,000,000 people. The rarity is further confirmed by the high incidence of consanguinity (42 per cent) among the parents of the patients. The best studied pedigrees are all typical of a recessive transmission. The step-wise enzymic breakdown of tyrosine through homogentisic acid to components of the citric acid cycle is known in detail, and the enzyme which normally oxidizes homogentisic acid is missing from the liver of an alkaptonuric patient.[12] Homogentisic acid therefore accumulates in proportion to the protein in the diet. It is excreted in the urine and causes the blackening already described. Many cases are still misdiagnosed at first as diabetics, because of the same confusion originally met by Bödecker one hundred years ago. The accumulation of homogentisic acid in the body tissues is small, but even that portion is also oxidized to the black polymer. Over the years this is selectively deposited in certain mesenchymal tissues; by middle age it results in the blackening of cartilage and tendons, known as ochronosis. This can be seen clinically as a bluish discoloration in the sclerotic coat of the eye, in ear cartilages and in tendons close to the skin. The single morbid consequence of the disease is the tendency of these dyed structures to break down, resulting in an incapacitating arthritis after middle age. This was undoubtedly the cause of the back pain of Bödecker's original case.

It is typical of the diseases of this group that they open new horizons to us. Still to be explored is the possible insight it gives us into the unsolved mechanism of arthritis. Thannhauser (1929) phrased this challenge provocatively:

> The arthritis of alkaptonuria is particularly instructive for general pathology, because we have here an endogenously produced metabolite, whose chemical structure is completely known, giving rise to a deforming joint disease. It is to take but one step to suggest that in the more common arthritis deformans of unknown etiology there is another endogenous metabolite acting like homogentisic acid and producing the joint changes.[17]

The close of Garrod's classical paper on "The Incidence of Alkaptonuria: A Study of Chemical Individuality" embodied a précis of the inborn errors of metabolism. He quickly sketched the salient characteristics of a new group of diseases, though only one example was known: A conspicuous and specific chemical deviation occurring among brothers and sisters, often the product of first cousin marriages. Albinism and cystinuria, he thought, might be other such diseases. In his Croonian lectures (1909) he marshalled the available evidence for this view, and added the fourth disease, pentosuria.

Universal Complete Albinism

While albinism undoubtedly fits into the group being discussed, our understanding of the chemical mechanism it involves is less complete than could be wished. We can not yet confirm Garrod's view that it is simply the lack of an

enzyme which usually makes pigment, and that the deficiency of the product rather than accumulation of the substrate as in alkaptonuria accounts for the major characteristics of the disease.[10b]

There are many kinds of pigmentary defects in man, sometimes associated with other severe anomalies, which often are not clearly distinguished from the characteristic albinism entity. Albinism is a simple condition: The lack of virtually all melanin pigment in hair, skin and eyes. The only complications, such as sunburn and abnormalities of vision, are those caused by inadequate shielding against light. Complete albinism quite precisely fits a recessive mode of inheritance. There is little evidence in man of the great variety of allelic* and non-allelic genes for albinism which occur in other animals.

Melanin, a brownish insoluble polymer of unknown chemical constitution, is formed in specific cells, the melanocytes. It probably is formed from tyrosine by tyrosinase. These melanocytes migrate from the neural crest during enbryological development to the sites which will be pigmented. Exclusion of these cells from a site results in localized albinism. W. K. Silvers (1957, 1958) has recently shown that the "clear cells" in the hair bulbs of albino mice are amelanotic melanocytes. But he also eliminated what evidence there was that (amelanotic) melanocytes are present in human albinos.[16] The best indication of their presence is the known occurrence of amelanotic melanomas in two human albinos. Upon this slender evidence rests the view that the defect is subcellular, possibly due to inactivity of tyrosinase. The histochemical "DOPA oxidase" tests are indeed negative in albino tissues. However, assay of the mammalian tyrosinase, which has "DOPA oxidase" activity, is complicated by the necessity of activating it for some days by irradiation or by removal of SH compounds. It is not yet certain whether the enzyme is absent in human albinos or whether some other subcellular abnormality prevents pigment formation.

Cystinuria

The third of Garrod's inborn errors of metabolism provided science with its first source of cystine in the urinary stones made of the pure substance characteristic of this disorder. Study of the patients also revealed the pathway of metabolism of sulfur-containing amino acids, even though there is no enzymic block in this metabolic pathway as Garrod assumed. A specific kidney transport system fails to conserve cystine as it normally does, and it is lost in the urine. This recent solution of the problem of cystinuria, assumed since Garrod's time to be a metabolic defect, forced an expansion of Garrod's original concept to include hereditary defects in other functional systems besides enzymes. It is also the only one of Garrod's four diseases whose hereditary transmission is not always purely recessive. About one-third of the families with cystinuria have a variety called "incompletely recessive", in which the carriers of a single cystinuria gene (the heterozygotes) can be distinguished by their excretion of moderately elevated amounts of

*One of a pair, or any one of a series, of genes having the same locus on homologous chromosomes.

cystine and lysine. These individuals account for the high incidence of "chemical cystinuria" (1:250) detectable by analysis, in contrast with the low incidence (1:80,000) of the homozygous excretors of gross amounts of cystine.[10]

In cystinuria of both varieties there is defective renal tubular reabsorption of cystine, lysine, arginine and ornithine. These amino acids, two of them more or less essential to the body, are excreted in the urine in abnormal amounts throughout life. Only the excretion of the least soluble one, cystine, was recognized until recent times, and both the name and the clinical importance of the condition is entirely referable to this one amino acid. The only clinical consequence appears to be the frequent formation of urinary calculi composed of almost pure cystine. Most individuals homozygous for the cystinuria gene sooner or later form such stones, with the stones tending to recur. The sequelae may well lead to eventual renal insufficiency and death.

Pentosuria

The last of Garrod's original inborn errors of metabolism is known only because the common tests for urinary sugar will detect it. Except for the first thirty or so cases known in Garrod's time that were imperfectly studied, and except for some minor and usually temporary excretions of various pentoses, almost all of the known instances of pentosuria conform to the characteristic pattern of a single disease entity. This condition is herited through a single autosomal* recessive gene. It is almost entirely limited to Jews, but a few patients of non-Jewish Mediterranean or European origin have been described. Its incidence is no greater than one to 50,000 in the general population, and probably quite high in a purely Jewish population.[10d]

The disease consists of the constant urinary excretion from infancy throughout life, almost independent of changes in diet, of about 2.0 to 3.5 g. per day of the reducing sugar, L-xylulose. There are no notable metabolic or clinical abnormalities, and the affected individuals have a normal life expectancy. In many cases neurasthenic symptoms have been prominent, but definite association of such symptoms with the disease has not been made. Often these are referable to the initial fright caused by suspicion of diabetes when urinary sugar was found.

Recent studies of pentose metabolism have revealed the reactions by which L-xylulose is formed and removed to be part of the metabolism of D-glucuronic acid. Hiatt (1958) found that labelled glucuronolactone did not give rise to labelled ribose in pentosurics as it did in normal controls. L-xylulose is normally reduced to xylitol, before conversion to ribose. Attempts by Touster and Harwell (1958) to localize the block before or after xylitol by measuring this sugar alcohol, resulted in the unexpected discovery of L-arabitol excretion in pentosurics. None of these results definitely prove that a block in metabolism exists, but if it does, it is likely the step forming xylitol. The alternate possibility of a renal mechanism to explain the excretion like that occurring in cystinuria has not been tested.

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*Any regular recessive gene as distinguished from a sex gene.

Other "Inborn Errors of Metabolism"

The second edition of Garrod's book added sketchy accounts of hematoporphyria congenita and of congenital steatorrhea to the four original diseases in this group.[4b] Subsequently it has been recognized that a number of other diseases also fit into the same general group, though few authors have had the temerity to label them explicitly as "inborn errors of metabolism." In truth the term is too restrictive. The modern view is that the original disease, as well as later ones of this group, can best be defined under a more general label such as the "hereditary molecular diseases."

None of the salient characteristics which allowed Garrod to single out his inborn errors of metabolism are essential qualities of these diseases. They need not be rare abnormalities — some hereditary chemical differences, such as blood groups, are now known to affect about half the population. They need not be recessive conditions—indeed it is being realized that any condition is "recessive" (unmanifested in the heterozygote with one abnormal gene) only if the basic molecular abnormality is not looked for. Garrod himself was aware that the abnormality need not be conspicuous. The striking abnormalities with which he dealt were simply the most obvious. Quite subtle differences now are regularly detected by the sophisticated analyses used in routine clinical work. And finally, as mentioned with regard to cystinuria, the primary defect need not involve an enzyme of metabolism. It appears that nearly any functional macromolecule, certainly nearly every functional protein, can be under hereditary control. There are literally thousands of unitary functions which make up the living processes of cells, and each is the role of a specific molecule. The truest statement of the present view is, one gene — one function. The scope presently envisaged for conditions of this sort encompasses most of the major and minor ways in which individuals differ from one another.

The Hereditary Molecular Diseases

We can now speak with some authority, although still only in general terms, about the molecular basis of the hereditary conditions which have been discussed. The gene, originally a hypothetical unit of inheritance, is a section along a helical tape of deoxyribonucleoprotein which makes up the chromosome of the cell. The essential features of this structure are the four bases — adenine, guanine, cystosine and thymine — arranged in a particular order along a simple chemical backbone. Using the initial letters of the bases, it is clear that a particular sequence such as — A - G - A - T - G - C — could represent coded information. The code has not yet been broken, but it is reasonable that particular sequences of three bases spell out each of the twenty amino acids used in protein synthesis. A gene, then, is a sufficiently long section of the tape to contain a "sentence" of such "words," a sentence which gives the amino acid sequence for a particular protein. A mutant gene has the sequence along the tape altered in some way. It is convenient to think of these tapes as analogous to the punched tapes on which information and instructions are fed into computors. Duplication of the tapes occurs in

cellular mitosis to pass on the information to later generations; and it is probable that "working copies" are made of ribonucleic acid for the actual job of protein synthesis in the cell.

It is no longer necessary to think of the synthesis of the specific proteins of the cell occurring by an infinite series of enzymes which make a protein, then by other enzymes which make the first enzymes, and so on. Only one general synthetic apparatus is required. It includes specific enzymes for carboxyl-activation with adenylic acid of each of the amino acids. Given the appropriately coded tape, and the stimulus for synthesis, this apparatus condenses the correct amino acids along the tape and then unzips them as a peptide chain of the appropriate amino acid sequence for the protein being synthesized. When the sequence of bases is altered in the gene, the sequence of amino acids in the protein is altered correspondingly. Usually the nature and function of the protein is affected. The often remote consequences to the organism of this altered structure and function of a protein molecule we see as the hereditary character or the disease picture. This view is amply supported by chemical evidence, but was best illustrated by the studies on another hereditary disease, sickle cell anemia.

Sickle Cell Anemia

The term "molecular disease" was introduced by Pauling (1955) when he and his associates showed that the hemoglobin of the red blood cells in this hereditary hemolytic anemia was chemically different from normal hemoglobin, and that all the signs and symptoms of the disease were referable to this molecular difference.[13] Ingram (1958) has identified this difference by "fingerprinting" on a chromatogram the digested fragments of the molecule.[7] A particular one of the three hundred amino acids in hemoglobin, one which is normally glutamic acid, is replaced by valine in the sickle cell hemoglobin. This does not significantly alter the oxygen carrying function of the hemoglobin, but the function in a larger physiological sense is seriously impaired. In its deoxygenated form, when it returns through the veins, the altered hemoglobin is very insoluble. It crystallizes inside the red cell, and distorts the cells to the elongated sickle shapes, giving the disease its name. These cells do not slide smoothly through the small vessels. They tend to clog and hemolyze and so produce the thromboses and anemia characteristic of the disease.

Findings of a similar nature have been made on several abnormal hemoglobins. Together they very clearly illustrate the genetic coding already described. The base sequence on the DNA tape describing hemoglobin has been altered so that valine replaces glutamic acid at one specific locus. The proof of this is still indirect. It is that the carrier of only one sickle cell gene and one normal gene does not have the overt disease, but about half of his hemoglobin is of the abnormal, valine-containing type. Under extreme conditions his red cells can be made to sickle moderately (the "sickle cell trait"), but this causes almost no clinical disability. With a double dose of the abnormal gene, nearly all of the hemoglobin is of the abnormal sickle type. It can be seen from family studies that the portions

of the abnormal molecule in hemoglobin-complement segregate just as do genes in the usual pedigree representation (see Figure 1).

The present picture, therefore, is very simple. An altered gene introduces a corresponding alteration in the amino acid sequence of the protein it produces. Two genes participate in the synthesis of the full complement of each protein; if both genes are altered, all the protein is abnormal. It remains to be shown that, like the hemoglobins, the protein parts of the inactive enzymes or the transport systems responsible for the hereditary diseases mentioned earlier are present in the cells, but altered in some specific way which makes them nonfunctional. In many instances the defect has been localized at least to the protein moiety. We cannot predict the exact functional effects of small alterations in protein structure, but we can expect some such changes to have slight effects and others severe effects. In this way we can account for the different alleles of genes whose effects are graded from mild to severe. We can also predict that hereditary abnormalities may exist which involve each of the many functional macromolecular compounds formed from genic patterns. It is also possible that some genic changes would make the coding into "nonsense", and no protein would be formed.

Phenylketonuria

This hereditary disease which results in mental deficiency currently is of great interest because dietary treatment can prevent its serious consequences. The disease is described here to emphasize a third general aspect of the hereditary diseases, the "pedigree of causes" which connects the inherited molecular abnormality with the ultimate consequences which are clinically significant. It has been emphasized that the hereditary conditions already mentioned have been life-long. But closer investigation reveals only the primary molecular disturbance to be life-long; the complications arising from it may depend on additional factors. Thus, a cystinuric patient daily excretes large amounts of cystine throughout his life, yet only a few times during his life will masses of cystine accumulate within his urinary tract to cause clinical symptoms. The alkaptonuric merely excretes a daily urine which blackens on standing. So trivial is this, many patients go through life without noticing the stain. Not until middle age does darkening of cartilages and stiffening of joints appear, which can be classed as disease, i.e., something which interferes with the well-being of the patient. Even the patient with sickle cell anemia has "crises" superimposed on a fairly normal life. In each of these examples, a genotypically and molecularly abnormal individual was apparently well except when some additional factors, acting with the basic abnormality, produced the overt disease signs and symptoms. Identification and control of these factors is the true province of scientific medicine and therapy. Phenylketonuria illustrates both these problems of disease mechanism and of therapy.

Phenylketonuria was discovered twenty-five years ago, and twenty-five years after Garrod's publication of the concept of the inborn errors of metabolism. It would be a typical example of this group, and indeed it was the first in which Garrod's postulated relation of *gene - enzyme - disease* was unequivocally demon-

strated. The exception to the usual picture that must be emphasized here is that the child who will have this disease is perfectly normal at birth — mentally, biochemically and molecularly.[11]

The uniform manifestations and precise identifiability of phenylketonuria allow it to be defined briefly and with unusual certainty. Both parents of a patient must have the defective form of one of the two genes controlling phenylalanine hydroxylase. The amount of phenylalanine hydroxylase activity in the parent is decreased enough so it can be detected chemically, but there is no serious consequence of this inadequacy. On the average, one out of four offspring from two such parents has both genes defective, has completely inactive phenylalanine hydroxylase, and has phenylketonuria. Both sexes are affected equally often. The disability is manifested only several weeks after birth, first with elevation of plasma phenylalanine to thirty times the normal level and the excretion of phenylpyruvic acid. After six months of age, retardation of mental development is noticed. Neurological abnormalities and seizures, diluted pigmentation of hair and skin, and eczema may develop. In older children and adults the process remains stationary. Life expectancy is seriously reduced, at least as much as in other forms of mental deficiency. The majority of patients are idiots, a few are imbeciles, and only rare patients are on the borderline of normal intellectual development. The incidence of this disease is about one in 25,000 among the mixed populations stemming from northern European countries. Most of the patients are segregated into institutions where they make up about one per cent of the mentally defective population. Patients are readily diagnosed by the olive-green color produced in the urine on addition of $FeCl_3$. This is due to phenylpyruvic acid, the typical metabolite of this disease.

A most surprising finding is that all infants at birth are like a phenylketonuric child in having no phenylalanine hydroxylase in their liver.[8] The enzyme normally appears first in the days or weeks immediately after birth in response to certain unidentified adaptive stimuli. This change is part of the normal biochemical defferentiation of the tissues. When phenylketonuric children are so stimulated, apparently only inactive enzyme is produced. The first point to be emphasized here is that a gene alone is not sufficient reason for protein formation. The genic information and the general synthetic apparatus to make phenylalanine hydroxylase was undoubtedly present in the normal fetal liver; this system, however, produced protein only when certain other conditions were met. Here is one possible explanation for those hereditary diseases which strike at certain ages, apparently for the first time. It is possible that until such an age, the defective gene had not been called into action.

The next point to be emphasized about phenylketonuria is the secondary nature of the mental defect. When phenylalanine hydroxylase fails to appear at the normal time, dietary phenylalanine in excess of needs for body building accumulates in the tissues and a variety of metabolites are formed in abnormal amounts. Yet this situation is not particularly harmful in itself. The infant remains well for several months, older children and adults have this abnormality

for years without showing signs of decline, and mothers with this condition have given birth to normal (necessarily heterozygotic) children. Only at a specific age, from about six months to four-plus years, is the child harmed by this metabolic abnormality. This critical period is also the important period for biochemical maturation of the brain, involving such complex reactons as myelination. It is thought that these processes may be peculiarly sensitive to the phenylalanine accumulation. The subtle disturbance of these critical processes at this time, in theory, causes the mental deficiency. Strong support for this view is offered by the growing number of children with normal intellect, even though they are phenylketonurics, whose dietary phenylalanine was therapeutically diminished during this critical period.

The third point about phenylketonuria is that dietary treatment is successful despite persistence of the genetic and molecular abnormality in the individual. It works simply by interrupting the causal chain which leads to the disease. In a sense, the disease of phenylketonuria, i.e., the mental deficiency, is acquired. It develops after birth and can be prevented. Only the primary molecular abnormality is inherited and persists. Except during a critical stage of brain maturation, it is a relatively harmless abnormality.

Heterozygosity

The fact was emphasized in connection with sickle cell anemia that the heterozygous carrier of one abnormal gene had about half his hemoglobin complement of the abnormal type. This is also true with respect to other varieties of abnormal hemoglobins. It is apparently true also for enzymic and other functional protein effects. The carrier of one type of cystinuria can be detected by his less than normal ability to resorb cystine, with consequent excretion of raised but clinically unimportant amounts of cystine. The carrier of one phenylketonuria gene has less than the normal amount of phenylalanine hydroxylase, as evidenced by his slow conversion of phenylalanine to tyrosine. This results in a higher than normal basal level of plasma phenylalanine, an unusually high and prolonged plasma elevation when given a test dose of phenylalanine, and a low level of tyrosine formed under the same conditions. Direct enzyme measurements in the blood cells of parents of galactosemic patients indicate that they too have less than the normal activity of galactose-1-phosphate uridyltransferase, the enzyme lacking in galactosemia.[9] Recent results, still unpublished (E. T. Nishimura), show very clearly the same picture in the families reported from Japan who have no blood catalase (acatalasemia). The heterozygotes have about half the normal activity. In a number of other hereditary diseases the heterozygotes can sometimes be identified by mild or minimal clinical stigma resembling the homozygous disease. As the primary molecular abnormalities in these conditions are identified and tests are devised to measure the functional molecules more or less directly, the list of detectable molecular abnormalities will grow in the apparently "normal" population.

The presence of abnormal molecules in heterozygotes of hereditary diseases is important for several reasons. As already discussed, it is important substantiat-

ing evidence for the one to one relation of the gene and the protein. Second, it can permit identification of the individuals who may have offspring affected with a serious disease, either to prevent this from happening, or to alert them to seek early therapeutic help for the children. Finally, such molecules are a very large source of the differences among individuals.

A few considerations about the population incidence of genes for even rare diseases will make it clear how in this way each member of the population can differ from his fellows. The number of heterozygous carriers of a gene for a rare disease is astonishingly high. It must be for even the few cases to be produced by chance matings in the population. A homozygous disease which occurs with a frequency of one in 40,000 people means

$$2\sqrt{\frac{1}{40{,}000}}$$

or one in 100 people must have one of these abnormal genes. There are easily more than one hundred such diseases, most of even higher incidence than one in 40,000. It has been estimated that each individual must therefore carry, on the average, half a dozen single genes, each of which in a double dose would result in serious disease. The point is not to raise fears about the quality of our hereditary material, but we should encourage every effort to safeguard it from further deterioration. The point is that each individual with an abnormal gene also has an abnormal type of molecule in his metabolic machinery, and will perform chemically in a subtly different way from many of his fellows. The fact people do differ from each other is the basis of much literature, but the attribution of these different personality traits, idiosyncracies, dietary requirements and disease susceptibilities to specific single gene abnormalities is only just beginning.

Dominance and Recessivity

Nearly everyone with a little genetic information attempts to fit hereditary conditions into the categories of dominant or recessive, or into intermediate degrees defined by the penetrance of the gene in question. It should be clear from this discussion that such categories are only descriptions of how particular characters are segregated in a family. In terms of molecules these words are meaningless. With one gene abnormal, about half the complement of the related protein is abnormal. Whether the disease is dominant, i.e., seen in an individual with one gene, or whether it is recessive and therefore manifested only when two such genes are present, will depend entirely upon secondary factors described under the pedigree of causes. The severity of the functional disturbance imposed by the single gene, the precision of the methods used in establishing the diagnosis, and the ubiquity of the environmental factors which operate as part of the pedigree of causes of ultimate clinical signs, these are the things which determine whether the hereditary disease pattern is dominant or recessive, not the gene-molecule relation. Molecularly, every hereditary abnormality is dominant.

Future Developments in Hereditary Disease

In only a few diseases, those chosen here as major examples, is a fairly complete picture available from the gene to the clinical signs. The purpose of the unified picture given here is to assimilate other hereditary conditions about which we have fewer facts, to give us a way of thinking about them and to guide further research on them. By way of summary, and somewhat speculatively, the possible relation of some other hereditary conditions can be outlined.

The group of hereditary morphological abnormalities have necessarily been omitted from the group discussed. This is not because structure is differently inherited than function, but simply because our knowledge of structure formation cannot yet be couched in molecular terms. The molecular reactions which go awry and produce six fingers, for example, are those occurring during embryological development. These are almost unknown to us, at least in this context. Like the phenylketonuric child who develops normally in nearly every respect except at a certain stage of brain maturation, the affected fetus may survive with only a structural abnormality to record the effects of a molecular imbalance at some critical period of its development.

There is much speculation whether some diseases of rather high incidence are hereditary, diseases such as diabebtes, rheumatoid arthritis, coronary heart disease and schizophrenia. In the picture of the hereditary molecular diseases a clear distinction is being drawn between the molecular abnormality which is inherited and the disease which develops from it. Most of the conditions studied so far have almost invariably resulted in disease. In these the pedigree of causes from molecule to clinical symptom was direct and little subject to variation. It is easy to imagine other molecular abnormalities which would cause trouble only in very particular situations. In these conditions environmental factors would be of paramount importance for the disease manifestation. Only a certain diet or a certain infection, for example, could set off the disease. The disease occurrence would therefore not conform exactly to genetic expectations. When the primary molecular difference is identified, this will be found to be inherited in a simple manner.

Another consequence of the dissociation of disease from the primary molecular alterations is that various molecular types can be accepted without calling them abnormal or defective. For example, the various blood group substances are inherited with exactly a one-to-one relation between gene and molecule. None of these are necessarily abnormal. Yet in certain conditions, like an Rh^+ fetus of an Rh^- mother or after a mismatched transfusion, even these commonly distributed molecules can cause trouble.

Emerging is a picture of a great variety of genes and therefore of molecules existing in the population. Largely unrecognized, these are the bases on which disease may develop, given the appropriate conditions. Contrariwise, given the gene, if the conditions can be controlled, the disease can be prevented. "The important thing to keep in mind in this connection is that genes are as amenable to modification of their consequences as any other factors . . ." [5]

Treatment

Hereditary disease is commonly viewed as a therapeutically hopeless situation. The possible modification of gene consequences belies this, and so do the remarkable instances of successful treatment which have been devised. Two main types of therapy have been successful: The alteration of cellular nutrition and the replacement of the disturbed functional molecule.

Cellular nutrition in a broad sense describes the biochemical environment of the cells. The important disturbances may either be the accumulation of an unmetabolized compound or a deficiency of a compound whose formation is blocked. Dietary galactose and phenylalanine restrictions effectively relieve the serious consequences of their accumulation in galactosemia and phylketonuria, respectively. There is hope exclusion of copper excesses will prevent Wilson's disease. Supplementation with a compound formed in deficient amounts is the basis of the effective therapy of the adrenogenital syndrome with corticoids and of certain familial cretins with thyroxine.

Replacement of the functional molecule is generally unsuccessful when this involves intracellular enzyme proteins. Yet the oxidation-reduction system which normally reduces methemoglobin to the functional form has been replaced by methylene blue in one form of methemoglobinemia. With two treatments a year these invalid children are restored to full activity. Hereditary inactivity of one of the plasma proteins is almost routinely treated by replacement. The various factors needed for blood clotting are known primarily because individuals lacking them were found, and treatment by transfusion is often life-saving during a bleeding crisis. The replacement of insulin in diabetes is so well known that it is often forgotten.

A third and most fertile avenue of therapy is still largely unexploited. This is the control of the environment of the individual as a whole and of his particular cells in such a way that molecular weaknesses do not cause disease. The chief limitation here appears to be our ignorance of disease mechanisms. In only a few instances is our knowledge adequate even to describe the problem. The child with agammaglobulinemia develops no resistance to infections. The infectious diseases are familiar hazards, so we do know how to go about safeguarding such a person. To prevent diabetes, coronary disease, schizophrenia, or even gout we must learn more about the molecular mechanisms of disease first revealed to us so clearly by the inborn errors of metabolism.

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